

An Update On
Chronic Health Effects Testing for Hydroquinone

Prepared For
Nonprescription Drug Manufacturers Association

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An Update On Chronic Health Effects Testing for Hydroquinone - 1

I. Executive Summary

In 1992, the NDMA reviewed the data available from numerous studies on the potential health impacts of hydroquinone (HQ) exposure. The conclusion reached based on this analysis of the then available data indicated that exposure to HQ in non-prescription lightening creams does not present a human carcinogenic risk when used according to label directions. At the time of the 1992, review, a research plan was outlined and initiated to provide additional information to more completely understand the significance of the test results for HQ. A particular focus of the research was to better understand the significance of the NTP bioassay results. The results of some of the studies incorporated into the 1992 plan are presented in this follow-up report which also includes preliminary information for on-going studies as well as outlines of studies which are in the planning phase. The results of the completed and ongoing research continue to support the conclusion reached in the 1992 review.

II. Index

	Page
I. Executive Summary	1
II. Index	2
III. Introduction	5
IV. Project Descriptions	9
Project I: Measurement of Cell Proliferation in the Kidneys of Fischer 344 and Sprague-Dawley Rats after Gavage Administration of Hydroquinone	9
Project II: Measurement of Nuclear DNA Modification by ³² P-Postlabeling in the Kidneys of Male and Female Fischer 344 Rats after Multiple Gavage Doses of Hydroquinone	11
Project III: Measurement of Cell Proliferation in the Kidneys of One Year Old Fischer 344 Rats after Oral Administration of Hydroquinone	13
Project IV: Quantification of Covalent Protein Adducts of Hydroquinone in the Blood and Kidneys of Fischer 344 and Sprague-Dawley Rats	15
Project V: Measurement of Oxidative Stress in the Kidneys of Fischer 344 and Sprague-Dawley Rats Given Hydroquinone	17
Project VI: A Comparative Study of the Enzymes Involved in the Bioactivation and Detoxication of HQ	19
Project VII: Comparative <i>In Vitro</i> Renal Metabolism and Toxicity of HQ Using Human and Rat Tissues and Cells	21
Project VIII: Development of a Physiologically-Based Pharmacokinetic Model for HQ	23

An Update On Chronic Health Effects Testing for Hydroquinone - 3

Project IX:	NDMA Formulation Cream: A Dermal Toxicity Probe Study in the Rat	25
Project X:	NDMA High HQ Formulation Cream: A Thirteen-Week Dermal Toxicity and Cell Proliferation Study in The Rat - Preliminary Results	28
Project XI:	The <i>In Vitro</i> Percutaneous Absorption of Hydroquinone from Aqueous Solution Through Rat and Human Skin	33
Project XII:	Human Exposure to Naturally Occurring Hydroquinone	34
Project XIII:	Epidemiology Investigation of a Hydroquinone/p-Benzoquinone Production Unit	36
V.	Conclusions	38
VI.	References	40

Appendices

- A. Measurement of Cell Proliferation in the Kidneys of Fischer 344 and Sprague-Dawley Rats After Gavage Administration of Hydroquinone
- B. Measurement of Nuclear DNA Modification by ³²P-Postlabeling in the Kidneys of Male and Female Fischer 344 Rats After Multiple Gavage Doses of Hydroquinone
- C. Measurement of Cell Proliferation in the Kidneys of One Year Old Rats After Oral Administration of Hydroquinone
- D. Quantification of Covalent Protein Adducts of Hydroquinone in the Blood and Kidneys of Rats
- E. NDMA Formulation Cream: A Dermal Toxicity Probe Study in the Rat

An Update On Chronic Health Effects Testing for Hydroquinone - 4

- F. Protocol for NDMA High HQ Formulation Cream: A Thirteen-Week Dermal Toxicity and Cell Proliferation Study in the Rat**
- G. The Absorption of Hydroquinone (HQ) Through Rat and Human Skin *In Vitro***
- H. Human Exposure to Naturally Occurring Hydroquinone**
- I. Mortality Study of Men Employed at a Large Chemical Plant, 1972 Through 1992**

**An Update On
Chronic Health Effects Testing for Hydroquinone**

III. Introduction

In 1992, the Non-Prescription Drug Manufacturers Association (NDMA) produced a review document on hydroquinone (HQ) which summarized the scientific literature available and outlined a research plan focused on increasing understanding of the potential for human health effects following HQ exposure. The finalized plan included 1) a survey of dermatologists to gather information on ochronosis, 2) an absorption and metabolism study in human volunteers, and 3) additional animal and non-animal testing on the mechanism(s) of action of HQ with the intent of developing a validated physiologically-based pharmacokinetic mechanistic model relevant to human health decision making. This update will focus on the results of the mechanistic research funded by NDMA and the Chemical Manufacturers Association Hydroquinone Panel and will not include information on Parts 1 and 2 of the research plan.

As discussed in the May 1992 review, for several years, HQ has been the subject of a series of health effects studies under a Toxic Substance Control Act Section 4 rule promulgated by the U.S. Environmental Protection Agency and a chronic toxicity and carcinogenicity bioassay conducted by the U.S. National Toxicology Program. These studies included endpoints for neurotoxicity, reproductive toxicity, developmental toxicity, genotoxicity, pharmacokinetics, chronic toxicity, and carcinogenicity. The results of these studies and many more in the published literature created a data base for HQ that is much more extensive than is available for many other chemicals.

Much of these data are derived from studies designed to maximize the likelihood of producing a toxic response by administering HQ in a high-dose bolus by the gavage route. While on the one hand such studies may be well designed for hazard identification, they have

An Update On Chronic Health Effects Testing for Hydroquinone - 6

the potential for producing data which are difficult to interpret for risk assessment purposes. This is particularly the case for materials such as HQ where the primary route of exposure is dermal and where dermal absorption does not result in significant toxicity.

The situation with HQ is more complex than simply trying to extrapolate data from studies using an oral route to a much more relevant dermal route of exposure, since toxicity related to specific species of animals or strains of rats can affect interpretation of the observations. Thus, additional data are being sought to more fully interpret the significance of these data for risk assessment purposes.

This research plan was designed to collect additional information to understand the significance of the available animal test results. The plan includes not only animal test data but also studies designed to collect relevant human data. Much of the plan has focused on increasing our understanding of the relevance of the increased incidence of benign kidney tumors observed in the male Fischer 344 rat during the NTP bioassay. As discussed in the 1992 review, the kidney tumors in the Fischer rat appear to be related to an increased susceptibility of the Fischer rat to HQ and in particular to an increased susceptibility of the male Fischer 344 rat to nephrotoxicity following chronic exposure to orally administered HQ.

To accomplish the purpose of this research plan, the studies discussed in the following pages were designed to:

Projects I and II: Determine if the nephrotoxicity observed in Fischer 344 male rats after oral HQ exposure is associated with either renal epithelial cell proliferation or DNA binding.

Project III: Determine if aging Fischer 344 rats were more or less susceptible to the nephrotoxicity associated HQ oral exposure.

An Update On Chronic Health Effects Testing for Hydroquinone - 6

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An Update On Chronic Health Effects Testing for Hydroquinone - 7

- Project IV:** Determine the extent and pattern of HQ-protein adducts in the blood and kidneys of Fischer 344 and Sprague-Dawley rats
- Project V:** Determine whether or not HQ exposure can produce oxidative stress in the kidneys of Fischer 344 and Sprague-Dawley rats
- Project VI:** Compare the activities of enzymes involved in the bioactivation and detoxication of HQ in Fischer 344 rats, Sprague-Dawley rats, and humans
- Project VII:** Compare the *in vitro* renal metabolism and toxicity of HQ using tissues and cells from Fischer 344 rats, Sprague-Dawley rats, and humans
- Project VIII:** Develop a physiologically-based pharmacokinetic model for HQ in sensitive and insensitive rat strains and humans
- Project IX:** Determine appropriate concentrations for a subchronic study of HQ with dermal application in a cream base
- Project X:** Determine whether or not HQ in a cream base when applied dermally is capable of inducing nephrotoxicity and cell proliferation in Fischer 344 rats
- Project XI:** Estimate the percutaneous penetration rate for HQ using an *in vitro* human *stratum corneum* and full thickness rat skin models
- Project XII:** Investigate potential dietary sources for exposure to naturally occurring HQ

An Update On Chronic Health Effects Testing for Hydroquinone - 8

Project XIII: Investigate the incidence of mortality due to cancer in a population of employees engaged in the manufacture of HQ and p-benzoquinone.

IV. Project Descriptions

Project I: Measurement of Cell Proliferation in the Kidneys of Fischer 344 and Sprague-Dawley Rats after Gavage Administration of Hydroquinone

Summary: Oral administration of hydroquinone (HQ) over two years to male Fischer 344 rats resulted in a dose-related nephropathy and an increase in the incidence of renal tubule adenomas (NTP, 1989). Female Fischer 344 rats, B6C3F1 mice, and Sprague-Dawley rats are refractory to the chronic renal toxicity of HQ. Dogs and humans are also refractory to nephrotoxicity following subchronic exposure (Carlson and Brewer, 1953). To better characterize the early development of renal toxicity in rats, cell proliferation was quantitated within the proximal (P1, P2, and P3) and distal tubule segments of the kidney in rats given 0, 2.5, 25, or 50 mg/kg HQ by oral gavage. Male and female Fischer 344 rats were treated for one, three, or six weeks and male Sprague-Dawley rats were treated for six weeks. Cell proliferation was quantitated by incorporation of bromodeoxyuridine, detected immunohistochemically, into newly synthesized DNA. At six weeks, renal cell proliferation was increased over vehicle-controls in male Fischer 344 rats dosed at 50 mg/kg. Significant elevations ($p < 0.001$) occurred in the P1 segments (87%) and in the P2 segments (50%) but the elevation in the P3 segment (34%) was not statistically significant. Urinalyses revealed increases in the rate of excretion of enzymes indicative of proximal tubular damage. Histopathologic evaluation of the kidneys was consistent with a dose-related tubular degeneration in the male Fischer 344 rat. No chemical-related effects were observed in the kidneys of female Fischer 344 rats and male

An Update On Chronic Health Effects Testing for Hydroquinone - 10

Sprague-Dawley rats. These data parallel the findings of sex- and strain-specific kidney adenomas in the two-year bioassays, and suggest that chemically-induced cell proliferation secondary to toxicity may be implicated in the etiology of benign tumors in the kidneys of male Fischer 344 rats treated with HQ.

Status: Project is completed and a report has been submitted to *Fundamental and Applied Toxicology* for publication. A full copy of the report is included in Appendix A.

Project II: Measurement of Nuclear DNA Modification by ^{32}P -Postlabeling in the Kidneys of Male and Female Fischer 344 Rats after Multiple Gavage Doses of Hydroquinone

Summary: Oral administration of hydroquinone (HQ) to male Fischer 344 rats results in dose-related kidney toxicity beginning with mild enzymuria by one week, significant cell proliferation by six weeks (see Project I), and nephropathy and an increase in the incidence of renal tubule adenomas after two years (NTP, 1989). Female Fischer 344 rats, B6C3F₁ mice, Sprague-Dawley rats, dogs, and humans are resistant to the renal toxicity of HQ associated with repeated exposure. To determine the potential of HQ to induce covalent DNA adducts in the kidney, male and female Fischer 344 rats were given 0, 2.5, 25 or 50 mg/kg HQ by gavage for six weeks, and nuclear DNA isolated from kidneys was analyzed by the ^{32}P -postlabeling assay. At 50 mg/kg, males, but not females, showed an increase in the rate of excretion of N-acetyl- β -D-glucosaminidase, indicative of proximal tubular damage. Analysis of nuclear DNA preparations by the postlabeling assay showed that HQ does not produce covalent DNA adducts in the kidneys of male and female rats. The assay's lower limit of detection is one adduct in 10^9 to 10^{10} DNA nucleotides. No treatment-related increases in background radioactivity levels on the chromatograms were seen at locations corresponding to the major *in vitro* adducts of HQ and *p*-benzoquinone. HQ treatment, however, resulted in the reduction of the levels of certain endogenous adducts (I-compounds), the biological significance of which is unknown. The results indicate that HQ does not produce covalent DNA adducts in the kidneys of male and female rats after repeated oral administration at nephrotoxic dose levels, and

An Update On Chronic Health Effects Testing for Hydroquinone - 12

suggest a nongenotoxic etiology of benign tumors in the kidneys of male Fischer 344 rats treated with HQ.

Status: **The project is completed and a report has been accepted by *Fundamental and Applied Toxicology* for publication. A full copy of the report is included in Appendix B.**

Project III: Measurement of Cell Proliferation in the Kidneys of One Year Old Fischer 344 Rats after Oral Administration of Hydroquinone

Summary: Oral administration of HQ to young adult Fischer 344 rats for 6 weeks produces mild kidney toxicity with elevated cell proliferation rates in males but not females. Chronic HQ administration results in an exacerbation of spontaneous nephropathy and an increase in the incidence of renal tubule adenomas, again in males but not females. Spontaneous nephropathy progresses more rapidly in male rats than in female rats, and may be a factor in the greater susceptibility of males to HQ-induced kidney effects. The present study was undertaken to measure HQ-induced cell proliferation in the kidneys of mid-life span Fischer 344 rats at dose levels that were tumorigenic in the males. Cell proliferation was quantitated within the proximal (P1, P2, and P3) and distal tubule segments of the kidney in male and female Fischer 344 rats (~ 13-15 months old) given 0, 2.5, 25, or 50 mg/kg per day HQ by gavage for three, six, or thirteen weeks. Cell proliferation was quantitated by incorporation of bromodeoxyuridine, detected immunohistochemically, into newly synthesized DNA. Urinalyses at 6 and 13 weeks revealed significant ($P < 0.01$) increases in the rate of excretion of N-acetyl- β -D-glucosaminidase by male and female Fischer 344 rats given 50 mg/kg per day when compared to concurrent controls, suggestive of mild kidney effects. However, histopathological evaluation of the kidneys was not able to differentiate HQ-induced effects from the background incidence of mild spontaneous nephropathy. Cell proliferation results showed no significant treatment related-effects. Mean cell proliferation rates in vehicle-control mid-life span rats were 2.2-fold lower than those observed in young adult animals. Subchronic exposure to HQ during mid-life is not sufficient

An Update On Chronic Health Effects Testing for Hydroquinone - 14

to exacerbate spontaneous nephropathy in male Fischer 344 rats.

Mid-life span male Fischer 344 rats are apparently more resistant to HQ-induced toxicity than their young adult counterparts.

Status:

A draft report is in preparation and an abstract of the study has been submitted for presentation at the 1994 Society of Toxicology meeting. A copy of the abstract is included in Appendix C.

Project IV: Quantification of Covalent Protein Adducts of Hydroquinone in the Blood and Kidneys of Fischer 344 and Sprague-Dawley Rats

Summary: In studies conducted by the National Toxicology Program (1989), hydroquinone (HQ) was found to increase the severity of chronic nephropathy seen in male Fischer 344 rats. In previous studies, HQ produced acute renal toxicity in Fischer 344 rats at overtly toxic oral doses. This same treatment had no significant effect on Sprague-Dawley rats. To investigate the importance of protein binding to the toxicity of HQ, a modification of the "permethylation" procedure of Slaughter and Hanzlik (1991) was employed to quantify HQ covalent adducts to protein sulfur nucleophiles. Male and female Fischer 344 rats and male Sprague-Dawley rats were dosed orally once with HQ at 0, 25, 50, or 100 mg/kg HQ and HQ protein adducts measured in blood and kidney 24-hr post dosing. Also, male Sprague-Dawley rats were dosed ip at equivalent dose levels. Separate groups of male or female Fischer 344 or male Sprague-Dawley rats were dosed orally for 6 wk (5 d/wk) at 0, 25, or 50 mg/kg/d and adducts measured 24-hr subsequent to final dosing. Following a single oral dose, female Fischer 344 rats were found to have the highest levels of bound HQ in both blood and kidneys at all dose levels. Levels found in the kidneys were on the order of 7 to 10 fold higher than for blood. The relative amounts of bound HQ in the rat followed the order found previously for acute renal toxicity: female Fischer 344 > male Fischer 344 > male Sprague-Dawley. Bound adducts in Sprague-Dawley rats following ip dosing were larger than the corresponding oral values. In all cases, total adducts increased proportionately with increasing dose level following a single administration of HQ. Animals dosed for 6 wk (max. 50 mg/kg/d) had quantitatively more protein bound HQ in both

An Update On Chronic Health Effects Testing for Hydroquinone - 16

blood and kidney with female Fischer 344 rats again having the highest levels. Levels found in the kidneys were on the order of 1.2 to 2.5 fold higher than for blood. The relative amounts of bound HQ in the 6-wk study followed the order found previously in the single dose study: female Fischer 344 > male Fischer 344 > male Sprague-Dawley.

Status:

A draft report is being prepared for submission for publication. An abstract of this work has been submitted for presentation at the 1994 Society of Toxicology meeting. A copy of the abstract is included in Appendix D.

An Update On Chronic Health Effects Testing for Hydroquinone - 17

Project V: Measurement of Oxidative Stress in the Kidneys of Fischer 344 and Sprague-Dawley Rats Given Hydroquinone

Summary: Experimental work sponsored by the CMA HQ Panel showed that a small proportion of an administered dose of HQ is metabolized to a compound that can be injurious to the kidneys of Fischer 344 rats. The means by which the HQ metabolite causes injury is unknown but is likely to involve protein binding (see Project IV) and/or the formation of reactive forms of oxygen. Previous work (Project II) established that HQ does not interact directly with kidney DNA *in vivo*. DNA could, however, be an indirect target if reactive forms of oxygen are formed that can subsequently enter the nucleus of renal epithelial cells and damage DNA. This type of oxidative damage has been shown to occur with HQ exposure *in vitro* and could possibly play a role in tumor development *in vivo*. Alternatively, reactive oxygen could be produced by a HQ metabolite without causing DNA damage. Knowledge of the extent of reactive oxygen formed in the kidney after HQ exposure, and the possible targets of any reactive oxygen formed (DNA vs. lipid), would help to elucidate the toxicological action of HQ in the Fischer 344 rat kidney. This information would also be useful in performing risk assessments for HQ exposure to humans. Several indicators of oxidative stress in the kidney which could result from the production of reactive oxygen by HQ will be measured in this study.

The test systems to be used include male and female Fischer 344 rats and male Sprague-Dawley rats. Rats will be given HQ by gavage at various dose levels and the time course of oxidative stress will be determined. Biochemical indicators of oxidative stress to be measured include an end-product of reactive oxygen reacting with lipid

An Update On Chronic Health Effects Testing for Hydroquinone - 18

(malondialdehyde), and the concentrations of glutathione and oxidized glutathione. Established spectrophotometric and chromatographic methods will be used to analyze the samples in these studies. Oxidative DNA damage will be measured in kidneys of rats treated for six weeks with HQ. The product of reactive oxygen reacting with DNA (8-hydroxydeoxyguanosine), will be measured with established chromatographic methods.

Status: The *in vivo* portion of this study is completed. The measurement of oxidative damage is underway.

Project VI: A Comparative Study of the Enzymes Involved in the Bioactivation and Detoxication of HQ

Summary: The observed differences in the susceptibility of different rat strains and species to HQ-induced kidney damage may be due to some combination of the following two factors; 1) pharmacokinetic differences, *i.e.*, differences in kidney concentrations of the injurious form of HQ based on differences in the rates of formation and elimination of the injurious form of HQ, and 2) differences in the inherent kidney sensitivity, *i.e.*, the same concentration of the injurious form of HQ may produce greater damage in a sensitive kidney compared to a resistant kidney. The former of these two factors will be addressed in this project. In understanding the relative risk of humans to the kidney effects of HQ, it is important to be able to understand the basis of the susceptibility of the male Fischer 344 rat to HQ. The activity and/or inducibility of HQ biotransformation enzymes (*e.g.*, quinone reductase, cytochrome P450s) may explain differences in urinary metabolites (and by inference, the concentrations of kidney metabolites) and the expression of kidney toxicity in the different test systems studied. This study will explore directly the enzymology of the relevant test systems, to determine if differences in enzyme inducibility or activity can explain the relative organism susceptibility by measuring the catalytic activities of several enzymes involved in the bioactivation and detoxication of HQ in susceptible and resistant strains of rats.

Biotransformations will be measured *in vitro* using various enzyme sources such as male and female Fischer 344 rat kidney, male Sprague-Dawley rat kidney, and human kidney. Biotransformations of the HQ-cysteine conjugate that will be measured include oxidation and N-

An Update On Chronic Health Effects Testing for Hydroquinone - 20

acetylation. The catalytic activity of γ -glutamyl transpeptidase and quinone reductase may be determined with the HQ-glutathione conjugate or other appropriate substrates. Concentrations of cytochrome P450s in kidneys will be determined by gel electrophoresis and Western blotting with immunodetection.

Status: Planning for this study is underway. The study is expected to be completed in 1994.

Project VII: Comparative *In Vitro* Renal Metabolism and Toxicity of HQ Using Human and Rat Tissues and Cells

Summary: The observed differences in the susceptibility of different rat strains and species to HQ-induced kidney damage may be due to some combination of the following two factors; 1) pharmacokinetic differences, *i.e.*, differences in kidney concentrations of the injurious form of HQ based on differences in the rates of formation and elimination of the injurious form of HQ, and 2) differences in the inherent kidney sensitivity, *i.e.*, the same concentration of the injurious form of HQ may produce greater damage in a sensitive kidney compared to a resistant kidney. The latter of these two factors will be addressed in this project. Obtaining human data is essential to assessing the health risks which might be associated with exposure to HQ. The use of an *in vitro* system is the best available system for collection of the relevant human data necessary for determining whether humans will respond more like susceptible or resistant experimental animals. In these studies, an *in vitro* system, either kidney slices or isolated kidney cells, will be used to examine the relative toxicities of HQ and HQ metabolites from various species and to determine sex, strain, and species (including human) susceptibility to HQ and its metabolites. The purposes of these studies are to 1) compare the relative toxicities of HQ and metabolites and 2) compare the responses of Fischer 344, Sprague-Dawley, and human kidneys to HQ metabolites. The toxicity of HQ and metabolites in the kidneys of resistant and susceptible test systems will be determined *in vitro* by exposing the test system to various concentrations of HQ, HQ-glutathione, HQ-cysteine, and possibly HQ-mercapturate and HQ-cys=O. Cytotoxicity will be assessed by established methods such as measurement of trypan blue exclusion or

An Update On Chronic Health Effects Testing for Hydroquinone - 22

lactate dehydrogenase leakage. Mitochondrial function, an early indicator of kidney toxicity, will be assessed by established methods including effects on adenine nucleotide status, cellular oxygen consumption, and cellular calcium compartmentation.

Status: Planning for this study is underway. The study is expected to be completed in 1994.

Project VIII: Development of a Physiologically-Based Pharmacokinetic Model for HQ

Summary: A physiologically based-pharmacokinetic (PBPK) model linked to an understanding of the mechanism(s) of action of HQ will be the ultimate end-product of this research program. PBPK models divide the body into physiologically realistic compartments and incorporate biologically correct mathematical descriptions of the major tissues and organs which are relevant to the absorption, metabolism, toxicity, and excretion of the chemical under study. The mathematical formulation of the model consists of a series of mass balance differential equations that account for the handling of the chemical by each compartment. PBPK modeling is a valuable tool for evaluating target tissue dose under various exposure conditions. Understanding target tissue dose is a necessary prerequisite for developing a full understanding of the biological responses associated with HQ exposure. The model will provide a construct for incorporating experimental data obtained in this research program and allow for the quantitative modeling of HQ behavior (*e.g.*, absorption, metabolic disposition, persistence) in various species including humans, at different exposure levels, and after various routes of exposure.

The model will be developed by acquiring, through the literature and independent research, physiological parameters including organ volumes and blood flows for the test systems of interest. Biochemical parameters including tissue:blood partition coefficients, kinetic constants for biotransformation, and macromolecular binding data will be incorporated into the model. The model will be validated with pharmacokinetic data obtained from the test systems of interest.

An Update On Chronic Health Effects Testing for Hydroquinone - 24

Human information may include blood and urine data from subjects who normally ingest fruits and grains which naturally contain HQ, as well as kidney autopsy specimens analyzed for stable HQ-protein adducts.

Status:

The data necessary for development of the PBPK model are being developed in Projects I-VII. The necessary hardware to build the model has been acquired and the software has been ordered. Model development will begin in 1994 as the data become available from Projects VI and VII.

Project IX: NDMA Formulation Cream: A Dermal Toxicity Probe Study in the Rat

Summary: Male and female Fischer 344 rats were topically treated with 0, 2.0, 3.5, 6.0, or 7.5% (w/w) of hydroquinone in an oil-in-water emulsion cream. Preparations were applied to the shaved skin of the back under a semi-occluded patch for 6 hr/day, 5 days/week for 19 doses over 24 days. Animals were observed daily for clinical signs of toxicity and signs of irritation. Body weights, feed consumption, and water consumption were measured at least twice weekly. No mortality occurred. Erythema was observed in all animals treated with hydroquinone, and the severity differed depending on the concentration in the preparation. Mean irritation scores of groups treated with 6.0 and 7.5% peaked at approximately 2 by the end of 4 weeks of dosing with maximum individual scores of 3; while the mean irritation scores of the group treated with 3.5% peaked at 1-1.5 with a maximum individual score of 2. The group treated with the 2.0% preparation had mean irritation scores of 1 and maximum individual values of no more than 1. Erythema progressively increased during the week with repeated application, but abated during the weekend when animals were not dosed. Irritation also increased slightly from week 1 to week 4 in the groups receiving the two highest concentrations. No edema was observed. Porphyrin tears and secretions were observed prior to dosing and were considered to be the result of wearing Elizabethan collars which were placed on the animals after dosing to prevent ingestion of test material during grooming.

No significant differences in mean body weight were noted among groups. Mean feed consumption by the 6.0% female group was

significantly ($p \leq 0.05$) lower on Day 4 compared with the control group. No other differences in mean feed consumption were noted among groups. Mean water consumption by the 2.0 and 3.5% male groups was significantly ($p \leq 0.05$) lower on Day 25 compared with the control group. Mean water consumption by all treated female groups was significantly lower on Day 14 compared with the control group ($p \leq 0.05$), and all treated female groups but the 6% group had significantly lower water consumption on Day 23 compared with the control group. Differences in water consumption were more apparent during dosing periods than on weekends.

After 24 days on test, the animals were anesthetized with carbon dioxide and exsanguinated. Treatment-related gross pathologic changes consisted of red and/or brown discoloration of the skin at the application site. Red discoloration was confined to the 7.5% male and female groups, the 6.0% male group, and one 3.5% female rat. Brown discoloration occurred at the application site of treated and some male control rats. Distinct brown discoloration was observed in the majority of hydroquinone cream treated rats. Sections of the skin at the application area, and an untreated section of skin from a more posterior region of the back, were preserved in 10% formalin and examined microscopically. Minimal to minor epidermal hyperplasia was observed at the application site of all treated groups, while minimal epidermal hyperplasia was observed at the application site of the control group. Hyperplasia at the application site in control animals is probably a reflection of repeated shaving as well as application of the vehicle. No histologic changes occurred in the untreated areas of skin of either treated or control groups. Minimal vascular congestion of the dermis was noted in an occasional animal treated with 6.0 or 7.5%

An Update On Chronic Health Effects Testing for Hydroquinone - 27

hydroquinone and in one 3.5% female rat. The vascular congestion roughly corresponded to an erythema score of 3 observed in these animals prior to necropsy. Additional compound-related changes included parakeratosis, acute inflammation, and vesicle formation in the dermis, all of minimal severity. No other treatment-related lesions were noted.

Based on the criteria for determination of the maximum tolerated dose (MTD), the MTD was not exceeded during the four weeks of dosing. Epidermal hyperplasia and parakeratosis were observed, and moderate erythema was present. However, the observation of erythema scores of 3 in male rats treated with 6.0 and 7.5% hydroquinone suggests that these concentrations might exceed the MTD during the course of the 13-week study.

Status: The final report is completed. Based on the results of this study, the high-dose concentration for the thirteen week study was set at 5% HQ. A copy of the report can be found in Appendix E.

Project X: NDMA High HQ Formulation Cream: A Thirteen-Week Dermal Toxicity and Cell Proliferation Study in The Rat - Preliminary Results

Summary: The following information should be considered preliminary results subject to revision as data are still be collected and quality assurance audits have not been completed on these data. Male and female Fischer 344 rats were topically treated with 0, 2.0, 3.5, or 5.0% (w/w) of hydroquinone in an oil-in-water emulsion cream. Preparations were applied to the shaved skin of the back under semi-occluded patch for 6 hr/day, 5 days/week for 13 weeks. Based on the concentrations of hydroquinone, the average weekly body weight, and the amount of material applied to the skin, treated male rats received doses of 29.5 ± 2.4 , 51.9 ± 4.3 , and 73.9 ± 6.3 mg/kg/day for the 2.0, 3.5, and 5.0% concentrations, respectively. Treated female rats received doses of 43.8 ± 3.8 , 77.0 ± 6.9 , and 109.6 ± 9.8 mg/kg/day for the 2.0, 3.5, and 5.0% concentrations, respectively. Animals were observed daily for clinical signs of toxicity. The only death observed occurred in one high-dose male that was found dead on the first day apparently due to the tightness of the wrappings. All other animals survived to their prescribed necropsy time. No compound-related signs of toxicity were observed other than local effects on the skin. Porphyrin tears and porphyrin nasal discharge were observed in all groups during acclimation to the collars and on mornings following the use of the Elizabethan collars, but were not observed on Sunday and Monday morning when collars had not been used. Compound-related clinical conditions consisted of minimal to minor brown discoloration and scaly skin at the application site. Brown discoloration occurred in all male groups, including the control group, and was observed in most animals

of each group after the first week of dosing. The severity was generally minimal and occasionally minor, and was observed daily. The frequency of minor brown discoloration versus minimal discoloration was greater in treated male groups compared with the control group, but there was no apparent dose-related response. In females, minimal brown discoloration of the skin was observed in only a few animals per group and only rarely in a control animal. Scaly skin (fissures) was observed in both male and female treated groups, and occasionally in a control animal. There was no apparent difference between the dose groups or between males and females.

Signs of dermal irritation were scored daily during Weeks 1, 2, 6, 7, 12, and 13 or once per week at other times. The erythema scores during Weeks 1 and 2 progressively increased during the week with repeated application, but recovered during the weekend when animals were not dosed. This pattern of daily irritation scores was repeated during Weeks 6, 7, 12, and 13. The maximum erythema score in any animal treated with HQ was 2 (well-defined erythema). Thus, based on the severity of erythema, the MTD was not exceeded. The maximum mean erythema scores in treated male groups were 1.3, 1.6, and 1.8 for the 2.0, 3.5, and 5.0% groups, respectively. In treated female groups, the maximum mean erythema scores were 1.5, 1.7, and 1.9 for the 2.0, 3.5, and 5.0% groups, respectively. Based on these data, females appeared to be slightly more sensitive to the irritation produced by the HQ preparations.

Body weights and feed consumption were measured at least once weekly. No significant differences in feed consumption were noted among groups except for Day 4 when the high- and mid-dose male

groups had significantly ($p \leq 0.05$) lower feed consumption compared with the control group. No significant difference in body weight was noted among groups.

Water consumption was measured three times a week during Weeks 1, 2, 6, 7, 12, and 13. Water consumption was, in general, comparable among groups. Mean water consumption values for male treated groups were generally within 10% of the values for the control group both during the week and on the weekends. Mean water consumption was 20-30% higher during the weekend compared with during the week; overall mean values for male rats during the week were 15.7, 15.8, 16.3 and 16.4 g/rat/day for the 0, 2.0, 3.5, and 5.0% groups, respectively, while overall means were all 20.6 g/rat/day during the weekend for these same groups. Overall mean values for female rats during the week were 13.4, 13.6, 13.6 and 13.7 g/rat/day for the 0, 2.0, 3.5, and 5.0% groups, respectively, while overall means were 17.1, 17.1, 17.3 and 17.6 g/rat/day during the weekend for these same groups. Slight differences in water consumption occurred from week to week with a general trend toward increased consumption over the course of the study.

Significant differences in water consumption between male treated and control groups occurred on Days 9, 37, and 88; however, these differences appear to be incidental and do not reflect an effect of the test substance. No significant differences were noted among female treated and control groups.

After 13 weeks on test, animals were fasted overnight, anesthetized, and blood collected for analysis. While significant differences occurred

in the hematology of male rats, none of the values appeared to be outside of the normal range of values for rats and were not considered to be biologically significant. No significant differences in hematology values were noted among female groups. Mean aspartate transaminase (AST), alanine transaminase (ALT), and sorbitol dehydrogenase (SDH) activities as well as total protein concentrations in the high-dose male group were significantly ($p \leq 0.05$) higher than in the control group. No significant differences in clinical chemistries were noted in female rats, however. Mean potassium concentrations in the high-dose male group were also significantly higher than in the control group. No changes occurred in sodium or chloride concentrations.

After blood collection, the animals were euthanatized and autopsied. The liver, kidneys, testes or ovaries, brain, lungs, adrenal glands, and thymus were weighed. No significant differences in absolute or relative organ weight were noted among groups. All tissues were preserved in 10% buffered-formalin. Selected tissues were examined histologically. The results of those evaluations are not complete.

During Weeks 3, 6, and 13, the satellite animals were anesthetized and Alzet® pumps containing BrdU implanted subdermally. These animals continued treatment with the test substance and at the end of three days were euthanatized. The carcasses were perfused with fixative, and the kidneys and duodenum excised. These tissues were embedded and stained for the presence of BrdU. Labelled cells were counted as an indication of cell proliferation. The results of these evaluations are not complete.

An Update On Chronic Health Effects Testing for Hydroquinone - 32

Status: The *in vivo* portion of this study has been completed. The urinalysis, histopathology, cell proliferation counting portions of the study are underway. Target completion date is January 1994.

Project XI: The *In Vitro* Percutaneous Absorption of Hydroquinone from Aqueous Solution Through Rat and Human Skin

Summary: The *in vitro* rate of percutaneous absorption of ^{14}C -hydroquinone was determined using full thickness rat skin and human stratum corneum. The rate of absorption from an approximately 5% aqueous solution of HQ for rat skin was found to be $1.09 \pm 0.65 \mu\text{g}/\text{cm}^2/\text{hr}$ ($n=5$), whereas the corresponding rate for human stratum corneum was $0.522 \pm 0.13 \mu\text{g}/\text{cm}^2/\text{hr}$ ($n=5$). The integrity of each skin specimen was determined by measuring the rate of penetration of $^3\text{H}_2\text{O}$ on the day preceding and the day following the HQ study. The "damage ratios" were near unity for both rat and human skin, indicating that little or no damage had occurred to the skin specimens from contact with the HQ test solutions. The *in vitro* permeability data for human stratum corneum are in reasonably good agreement with *in vivo* permeability data reported by Bucks *et al.* (1988) of $3.0 \mu\text{g}/\text{cm}^2/\text{hr}$. The permeability constant (K_p) for HQ through human stratum corneum was calculated to be $9.58 \times 10^{-6} \text{ cm}/\text{hr}$. Using the definitions of Marzulli, Brown, and Maibach (1969), HQ would be classified as a "slow" permanent for human skin.

Status: The results of this study were presented at the 1993 Society of Toxicology meeting. A copy of the final report can be found in Appendix G.

Project XII: Human Exposure to Naturally Occurring Hydroquinone

Summary: Hydroquinone (HQ) is a nonvolatile chemical used in the photographic, rubber, chemical, and cosmetic industries. HQ is also known to occur in nature as the β -D-glucopyranoside conjugate (arbutin), and free HQ is a known component of cigarette smoke. Low concentrations of HQ have been detected in the urine and plasma of humans with no occupational or other known exposure to HQ. This study investigated dietary and other potential sources of HQ and their influence on HQ concentrations in the plasma and urine of humans. Analysis of possible food sources of HQ by gas chromatography indicated significant amounts of arbutin in wheat products (1-10 ppm), pears (4-15 ppm), and coffee and tea (0.1 ppm). Free HQ was found in coffee (0.2 ppm), red wine (0.5 ppm), wheat cereals (0.2-0.4 ppm), and broccoli (0.1 ppm). After consuming a meal including arbutin- and HQ-containing foods, humans showed significant increases in plasma and urinary levels of HQ and its conjugated metabolites (total HQ). Mean plasma concentrations of total HQ peaked at 11x background levels at 2 hrs after the completion of the meal, and urinary excretion of total HQ peaked at 16x background amounts at 2 to 6 hrs after the meal. Immediately after smoking 4 cigarettes, mean plasma concentrations of total HQ were maximally 1.5x background levels; urinary excretion of total HQ peaked at 3x background amounts at 1 to 3 hrs after smoking. These data indicate that appreciable human exposure to HQ can result from plant derived dietary sources of the chemical and to a lesser extent, from cigarette smoke.

An Update On Chronic Health Effects Testing for Hydroquinone - 35

Status: A draft report is being prepared. An abstract of the results of this study has been submitted for presentation at the 1994 Society of Toxicology meeting. A copy of the abstract can be found in Appendix H.

**Project XIII: Epidemiology Investigation of a Hydroquinone/p-Benzoquinone
Production Unit**

Summary: A mortality study (Appendix I) of a large chemical production facility, including a population engaged in the manufacture of HQ, was completed in 1986. The Organic Chemicals Division which included the HQ unit had a significantly lower number of total deaths than expected and the Standard Mortality Ratio for all malignant neoplasms in these employees was 56.

The current epidemiologic study has been designed to (1) evaluate the mortality experience of a cohort of employees from the same plant studied in 1986 who were involved in HQ manufacturing and related activities, (2) test hypotheses for deaths due to renal and hepatic tumors and leukemia, and (3) assess dose-response relationships for these hypothesized cancer sites according to estimated career HQ exposure and time-from-first exposure (latency).

The study population, identified from clinical examination lists, medical records, and computer files, will include about 900 men and women who worked at least six months between 1930 and 1990 in HQ production operations. The vital status of the subjects will be obtained from both internal (company) and external (Social Security Administration) sources. The underlying cause of death will be coded by a nosologist according to the International Classification of Diseases (ICD-9) protocol.

The mortality experience of cohort members will be compared with death rates from both general population and two occupational referent

An Update On Chronic Health Effects Testing for Hydroquinone - 37

groups. The statistical significance of observed-to-expected differences will be tested according to two sets of criteria, depending upon whether the cause of death was hypothesized or non-hypothesized.

Estimates of HQ exposure will be derived from air sampling analyses and from occupational histories abstracted from employee records. Each of the jobs held by an individual throughout his career in HQ operations will be assessed in terms of its duration (months) and estimated exposure level. The product of these values will be accumulated for each study subject and converted to total career exposure for analytical purposes.

Status: The mortality data for the study have been compiled and are undergoing analysis. The study is expected to be completed in the first quarter of 1994.

V. Conclusions

The research program which was outlined in the May 1992 NDMA report is progressing according to plan and has produced results which support the hypothesis that the benign kidney neoplasms seen in male Fischer 344 rats are due to an unusual sensitivity of this animal model to hydroquinone (HQ).

HQ is a part of the normal diet of humans and can be found in foods and beverages of plant origin. Whether from natural sources (fruits and vegetables) or from synthetic sources (Carlson and Brewer, 1953), HQ is not expected to result in the same type of toxicity that is seen in the Fischer 344 rat.

The research results demonstrate that the male Fischer rat develops a toxic nephropathy which is unusual in that male Sprague-Dawley rats given similar doses of HQ do not demonstrate a similar toxic effect and female Fischer rats while susceptible to developing nephropathy are not as susceptible as the males and do not develop benign renal neoplasms after long term HQ exposure. The nephropathy which is seen in the male Fischer 344 rats is associated with an increase in the rate of renal epithelial cell proliferation but is not associated with the formation of DNA adducts in the target cell population. At dose levels which are nephrotoxic, HQ binding to renal cellular proteins can be observed. In this nephropathy, protein binding may play a role in the pathogenesis of renal damage as Sprague-Dawley rats show less protein binding than male Fischer 344 rats when dosed orally but show much higher levels of protein binding when they are dosed by a route of administration (ip) which overwhelms their normal defense mechanisms and induces nephropathy. A number of additional studies are underway to enhance our understanding of the mechanisms by which HQ is associated with renal tumor formation in the male Fischer 344 rat.

An Update On Chronic Health Effects Testing for Hydroquinone - 39

Of more direct importance to the risk assessment process are data which demonstrate that HQ is only slowly absorbed through rat and human skin. The dermal toxicity and cell proliferation study with Fischer 344 rats which is underway will provide important information about whether or not dermal HQ exposure can be associated with renal effects in this sensitive model of nephropathy.

The data collected in this research program support the conclusion made in the 1992 NDMA report that HQ in nonprescription skin lightening creams do not present a human carcinogenic risk when used according to label directions.

VI. References

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